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Evaluation of antiasthmatic activity of *Caesalpinia bonducella* [L.] Roxb. seed

Prakash D. Khandagale *, Abhijeet V. Puri

Department of Pharmacognosy, St. John Institute of Pharmacy and Research, Vevoor, Manor Road, Palghar (E), At Post. Tal / Dist: Palghar-Pin-401404 (MS)

ABSTRACT

Objective: The study was planned to investigate the antiasthmatic activity of *C. bonducella* seeds by means of various *in-vitro* and *in-vivo* animal models.

Methods: In the present study, petroleum ether and ethanolic extracts of *C. bonducella* seeds were evaluated for preliminary phytochemical screening and antiasthmatic activity *in-vitro*.

Results: Preliminary phytochemical screening has revealed the presence of steroids, saponins, flavonoids, alkaloids, and tannins. Ethanolic extract of *C. bonducella* seeds exhibited antihistaminic activity at the dose of 50 and 100 mg/kg, it inhibited clonidine-induced catalepsy but not haloperidol-induced catalepsy. Ethanolic extract significantly inhibited increased leukocyte besides eosinophil count due to milk allergen moreover showed maximum protection against mast cell degranulation by clonidine. The results of guinea pig ileum indicated that the compound 2-methyl, 1-hexadecanol isolated from ethanolic extract significantly relaxed the ileum muscle strips that were pre-contracted.

Conclusion: The present study concludes that the antiasthmatic activity of ethanolic extract *C. bonducella* seeds may be due to 2-methyl, 1-hexadecanol. Hence, a detailed study needs to be conducted to isolate phytoconstituents responsible for antiasthmatic activity accordingly their clinical efficacy in the treatment of related diseases.

Keywords: *C. bonducella,* Phytochemical Screening, Antiasthmatic activity

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***Address for Correspondence:**

Prakash D. Khandagale, Department of Pharmacognosy, St. John Institute of Pharmacy and Research, Vevoor, Manor Road, Palghar (E), At Post. Tal / Dist: Palghar-Pin-401404 (MS)

INTRODUCTION

Asthma can be characterized by increased sensitivity of trachea and bronchi to innumerable stimuli and exhibited by severe, intermittent, and chronic attacks of extensive contraction of airways. Clinically it is expressed by obstruction of the airway that encompasses inflammation of the pulmonary airways and respiratory hyper responsiveness that is commonly reversible.[1] Asthma can be caused by allergic hypersensitivity reactions that do not respect the borders of age, race, gender and several deaths are reported to be occurring annually.[2] Genus of *Caesalpinia* is widespread in 500 species that has medicinal benefits based on their pharmacological activity. One of the medicinal plants from this genus is *Caesalpinia bonducella.* Linn (*C. bonducella)* an Indian herb belonging to Family *Caesalpinia*ceae. It is found throughout India and other tropical countries of the World. Name '*Bonducella*' of the species is derived from the Arabic word "Bonduce" which means a "little ball" that indicates the globular shape of the seed. [3-4] Literature review marked the presence of some imperious phytochemicals such as bonducellin, phytosterinin, β-sitosterol, furanoditerpenes, flavonoids, aspartic acid, arginine, citrulline, β-carotene.[5] The seed kernel of plant *C. bonducella* essentially contains bonducin, sulphur-containing compounds and seed moreover comprise of unsaturated fats.[6-7] *C. bonducella* seeds also contain alkaloid as caesalpinine, and bitter principles such as bonducin.[8] Triterpenoid, fatty acid triglycerides, and sterols isolated from seeds may possibly render herb its therapeutic properties.[9-10] In view of above-mentioned scientific pieces of evidence and uses of *C. bonducella* L. endeavor was made to investigate the antiasthmatic activity of seed extract of *C. bonducella* L.

MATERIALS AND METHODS

Procurement of plant materials:

The *C. bonducella* seeds collected from neighboring cantonment region of Sangamner, Ahmednagar district. Seeds were then cleaned and dried in shade at room temperature, exposure to direct sunlight was avoided. Authentication and botanical identification of the plant material were carried out by Dr. T. Chakraborty, Joint Director, Botanical Survey of India, Pune. A voucher

specimen of a plant is deposited for future reference (Voucher Specimen Number: BSI/CAEB7PRAK)

Drying and Size reduction

The seeds of *C. bonducella* were cleaned to clear the adhered foreign material and were washed beneath tap water, air dried, homogenized to powder and stored in hermetically sealed bottles.

Procurement of Chemicals

All the chemicals utilized were of analytical grade and were procured from Merck, India, PCL, Sunpharma, Unichem, Lupin, Unimark Remedies Pvt Ltd. India.

Phytochemical Screening

The seeds of *C. bonducella* were procured, dried in the shade and subsequently powdered in a homogenizer. The powdered seeds were used for extraction. Powder drug was passed through 120 mesh to remove the fine powder. Coarse powder material (500g) was subjected to successive solvent extraction with petroleum ether and 70% ethanol in water in Soxhlet apparatus. Petroleum ether and the ethanolic extract was concentrated below reduced pressure using a rotary evaporator and dried in vacuum and subjected to preliminary phytochemical screening.[11-15]

Experimental Animals

Male Swiss mice (20-25 g) and Male guinea pigs (250-350 gm) were accommodated under standard laboratory conditions with free access to food and water. The experimental protocol was approved by the animal ethical committee of the institute (Approval No. 448/01/C/ CPCSEA/10-11/04).

Acute Toxicity Testing

Petroleum ether and Ethanolic extracts of *C. bonducella* seeds were tested on adult albino mice of either sex were separated into nineteen groups (n=6 in each group). Each mice from individual groups fasted for 18 hrs. and was administered with varying doses of extract (800,900,100, 1100, 1200 and 1300 (mg/kg, i. p.). Control group of mice were administered with 5% tween 80 solution in distilled water. Individual mice were practically observed for 3days, and the LD_{50} was calculated. [16-17]

Effect on clonidine-induced catalepsy

Bar test was utilized to evaluate the impact of different extracts on clonidine-induced catalepsy. Mice were isolated into 10 groups. Clonidine (1 mg/kg, s.c.) was administered to mice (n = 6) pretreated 30 min. earlier with vehicle (5 ml/kg, i.p.), petroleum ether and ethanolic extracts (50 and 100 mg/kg, i.p.) and Chlorpheniramine maleate (10 mg/kg, i.p.). The forepaws of mice were held on the flat bar and the time required to uplift the paws of the bar was noted. This was performed with individual and the duration of catalepsy was determined. [18]

Effect on haloperidol-induced catalepsy

In similar bar test mice (n = 6) was administered with Haloperidol (1 mg/kg, i.p.) which were pretreated 30 min earlier with vehicle (5 ml/kg, i.p.), petroleum ether and ethanolic extract (50 and 100 mg/kg, i.p.). The extents of catalepsy were determined. [19-20]

Milk-induced leucocytosis and eosinophilia in mice

Mice were distributed into six animals in each of six groups. Blood samples were withdrawn through retro-orbital plexus. Total leukocyte and eosinophil count was determined for each group before treatment of drug and after 24hrs of boiled and cooled milk injection. Group I was treated with vehicle. Group II received vehicle and milk (4 ml/kg, s.c.). Group III to VI were injected with petroleum ether and 70% ethanolic extracts (50 and 100 mg/kg, i.p.), respectively and after 30 min of drug treatment each animal was administered with milk (4 ml/kg, s.c.). Difference in total leukocytes count and total eosinophil count before and after 24 hr. of drug treatment was determined. [21-22]

Mast cell stabilizing activity

Mice were divided into six groups, six animals in each group. A three day drug treatment schedule was followed. Group-I received vehicle (5 ml/kg, i.p.). Group-II was injected with standard disodium chromoglycate (200 µg/kg. i.p.). Group III to VI were administered with petroleum ether and 70% ethanol extracts (50 and 100 mg/kg, i.p.), respectively. On the fourth day each animal was administered with 4 ml/kg, 0.9% of saline solution into peritoneal cavity. Later peritoneal fluid was withdrawn through gentle massage after 5 min. and added into siliconised test tube containing 7-10 ml RPMI 1640 buffer solution (pH 7.2-7.4) and centrifuged 400-500 rpm. After centrifugation pellets of mast cells were treated twice with similar buffer solution and supernatant was discarded. These cells were tested with clonidine (50 ug/ml) and incubated in a water bath at 37^{0}C for 10 min which was then stained with 1% toludine blue. Later it was observed under microscope at (45x) and percent protection contrary to degranulation was determined.[23]

Studies on smooth muscle preparation of guinea pig ileum (*in-vitro*)

The guinea pigs (overnight fasted) were sacrificed by a sharp blow over the head, abdomen was opened and ileum was dissected and mounted in an organ bath containing Tyrode solution, which was uninterruptedly aerated at 37±0.5°. Dose response curve of histamine in plain Tyrode solution and in Tyrode solution containing 100 µg/ml seed extract were performed. After acquiring a dose response curve petroleum ether extract (100 µg/ml) was added to the reservoir containing Tyrode solution and a response of tissue was recorded for the same dose of Histamine to obtain inhibition curve. After subsequent washings 70% ethanolic extract was added to the reservoir same procedure was repeated to obtain inhibition curve. Percent maximum relaxation response versus concentration of histamine was plotted to record dose response curve of histamine in presence of both the extracts. [24-25]

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. The values are expressed as mean ± SEM and $P<0.05$ was considered significant.

RESULTS & DISCUSSION

Phytochemical Screening

The qualitative phytochemical screening was carried out to detect presence of phytoconstituents in various extracts. Phytochemical screening confirmed the presence of carbohydrates, alkaloids, flavonoids, triterpenoids, proteins, saponins, steroids, tannins and glycosides. Phytochemical screening indicates that *C. bonducella seeds* contain different polar and non-polar phytochemicals present in the drug shown in Table 1.

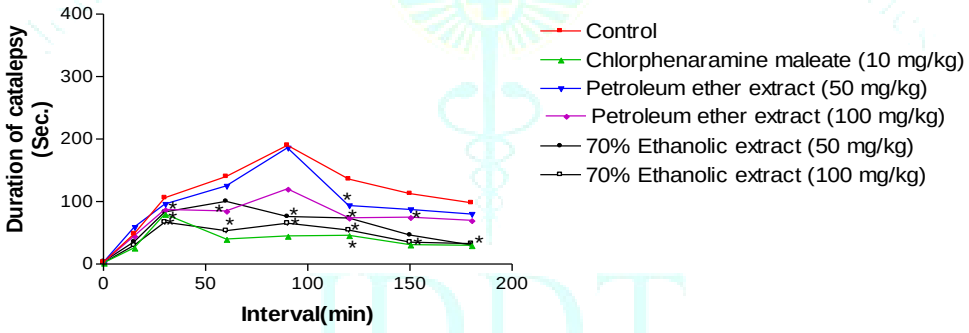
Table 1: Qualitative phytochemical screening of ethanolic extract of *C. bonducella seed.*

Phytochemicals test	Pet-ether Extract	Ethanolic Extract
Alkaloids	-	+
Dragendroff's test	-	+
Mayer's test	-	+
Wagner's test	-	+
Hagers's Test	-	-
Proteins and Amino acids	-	+
Flavonoids Alkaline reagent	-	+
Flavonoids Shinoda Test	-	+
Tannins FeCl3 test	-	+
Phlobatanins Hcl test	-	+
Triterpenes H2SO4 test	+	-
Steroids Liebermann and Burchard test	+	-
Saponins Frothing test	+	-
Cardiac glycosides Keller-kilianni test	-	-

+: indicates presence of constituents -: indicates absence of constituents

Clonidine Induced Catalepsy in Mice

The results indicated that the 70% ethanolic extract exhibited antihistaminic activity, inhibited clonidine-induced catalepsy but did not inhibit haloperidol-induced catalepsy. The cataleptic effect of clonidine on the mice is triggered by histamine release from mast cells. The effect of the extract on clonidine-induced catalepsy showed the extent of catalepsy in the petroleum ether extract treated group was considerably reduced in comparison to the control group. Clonidine-induced phase of catalepsy in mice up to 140.3±0.4 s. The impact of ethanolic extract was found to be more noteworthy (P<0.05) in reducing the clonidine-induced catalepsy compared to petroleum ether extract. The treatment of Chlorpheniramine maleate (10 mg/kg, i.p.) has exhibited clonidine-induced catalepsy. Reduction of catalepsy is found to be a dose-dependent with ethanolic extract as shown in Figure 1:



*P<0.05 compared to vehicle treated group (One way ANOVA and Dunnett's test)

Figure 1: Effect of various extracts of seeds of *C. bonducella* on clonidine induced catalepsy in mice.

Haloperidol Induced Catalepsy in Mice

The results indicated that the ethanolic extract exhibited antihistaminic activity, inhibited clonidine-induced catalepsy but did not inhibit haloperidol-induced catalepsy as illustrated in Figure 2. Haloperidol lengthened degree of catalepsy up to 89.38±0.1 s. In comparison to standard drug Buspirone the extracts have considerably reduced the haloperidol induced catalepsy.

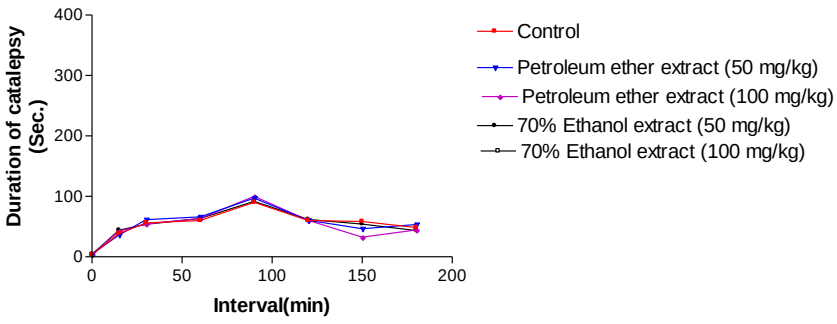
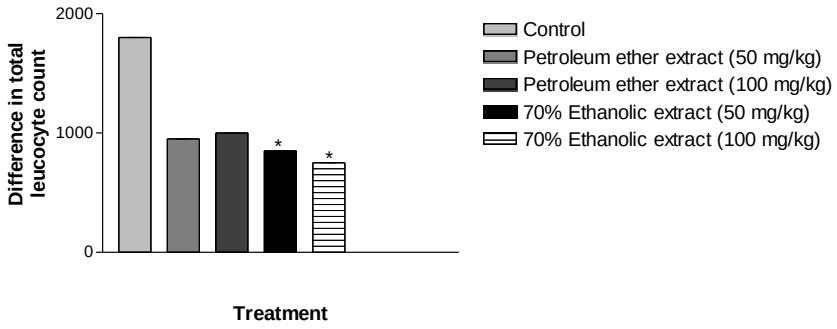


Figure 2: Effect of various extracts of seeds of *C .bonducella on* Haloperidol induced catalepsy in mice.

Milk Induced Leukocytosis in Mice

Control group showed extreme increase in leukocytes count 24 h on subsequent administration of milk (4ml/kg, s.c.). Ethanolic extract inhibited increase in leukocyte count (P<0.05) owing to milk allergen in comparison with petroleum ether extract. The decrease in leukocyte count with ethanolic extract was found to be dose dependent shown in Figure 3. Thus 70% ethanolic extract exhibited protecting effect against milk-induced leucocytosis.

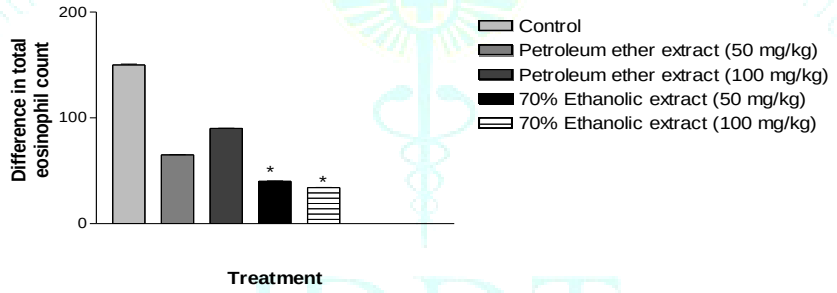


All the data is expressed as mean ± SEM, n=6 in each group.*P<0.05 compared to control group.

Figure 3: Effect of *C. bonducella* seed extracts on milk-induced Leukocytosis in mice.

Milk Induced Eosinophilia in Mice:

Control group showed an extreme increase in eosinophil count 24 h on subsequent administration of milk (4ml/kg, s.c.). Ethanolic extract inhibited the increase in eosinophil count (P<0.05) owing to milk allergen in comparison with petroleum ether extract. The decrease in eosinophil count with ethanolic extract was found to be dose-dependent shown in Figure 4. Thus 70% ethanolic extract exhibited protecting effect against milk-induced eosinophilia which is valuable as antiallergic in asthmatic condition.

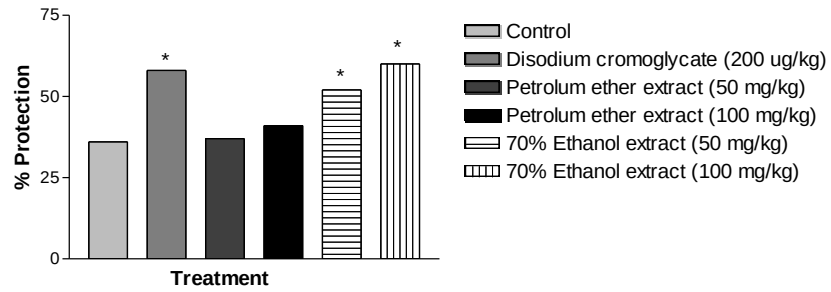


* All the data is expressed as mean ± SEM, n= 6 in each group.*P<0.05 compared to control group.

Figure 4: Effect of *C. bonducella* seed extracts on milk-induced eosinophilia in mice.

Clonidine induced mast cell degranulation in mice:

Degranulation of mast cell is observed when treated with clonidine as seen in control group. Ethanolic extract considerably reduced degranulation of mast cell by clonidine in comparison to other extracts. These outcomes are comparable with disodium chromoglycate (200ug/kg, i.p.). 2 methyl, 1 hexadecanol that protects mast cell degranulation as that of protection showed by disodium chromoglycate. The results are noteworthy (P<0.05) compared with the control group and found to be dose-dependent shown in Figure 4 and Figure 5.



All the data is expressed as mean ± SEM, n= 6 in each group.*P<0.05 compared to control group.

Figure 4: Effect of *C. bonducella* seed extracts on mast cell stabilizing activity.

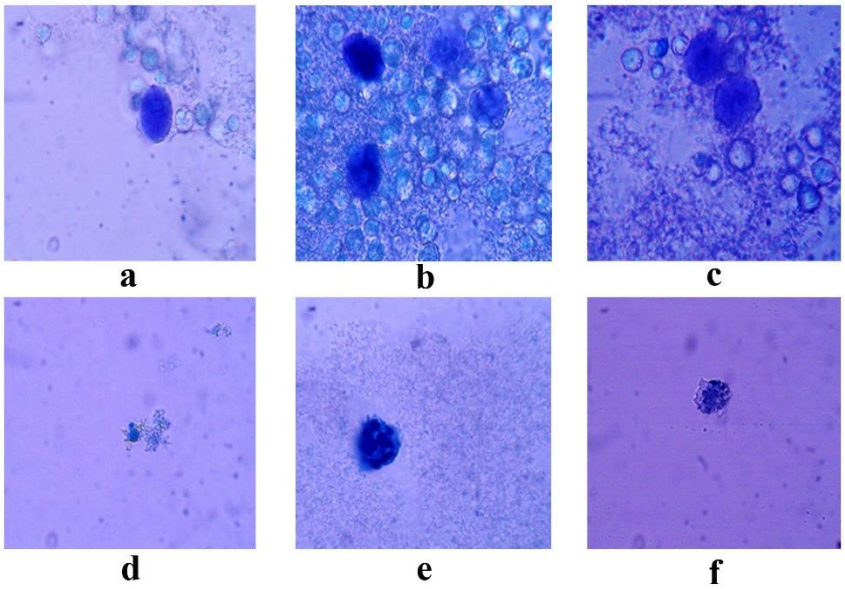


Figure 5: Histopathology of Mast Cells

a) Non granulated mast cell b) Granulated and non-granulated mast cells
c) Partially granulated cells d) Mast cell with degranulation
e) Incomplete Granulation f) Incomplete granulation

Studies on smooth muscle preparation of guinea pig ileum (*in vitro*)

The result indicated that the 70% ethanolic extract considerably relax the ileum muscle strips pre-contracted by treatment of histamine showed in Table 2. This outcome suggests an association of β2-agonists on the relaxation of the tissue. The capability of the 70% ethanolic extract to inhibit the contraction induced by the bronchoconstrictor histamine proposes a potential role in the treatment of asthma. Furthermore, the relaxation of histamine pre-contracted ileum by extracts indicates their potency in ameliorating established asthma. This study also showed anticataleptic, antiallergic, mast cell stabilizing and a relatively potent relaxant (bronchodilator) effect of 70% ethanolic extract on the tracheal chain of the guinea pig and low toxicity shown in Figure 6.

Table 2: Effect of Histamine on Isolated guinea pig ileum

Treatment	Histamine(ug/ml)	% Relaxation
70% Ethanol extract (100 ug/ml)	0.1	100
	0.2	77.43
	0.3	52.52
	0.4	42.86
Petroleum ether extract (100 ug/ml)	0.1	85
	0.2	66.67
	0.3	57.15
	0.4	52.39

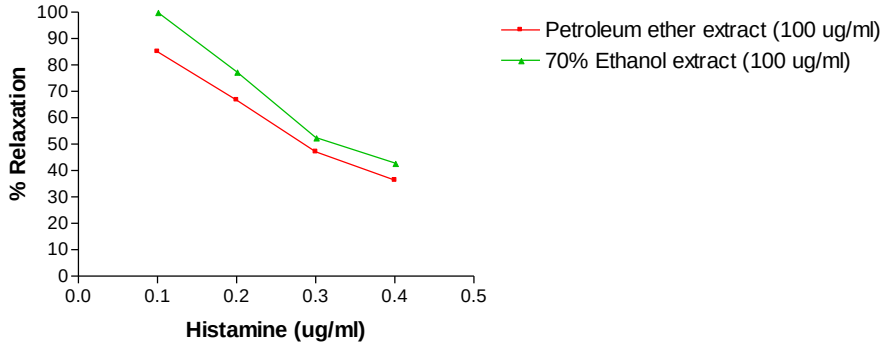


Figure 6: Effect of various extracts of *C. bonducella* seeds on contractile response of histamine on isolated guinea pig ileum.

CONCLUSION

The present work was embraced with a viewpoint to set down benchmarks which could be valuable in recognizing the authenticity of this medicinal herbal. Phytochemical screening showed the presence of steroids, saponins, flavonoids, alkaloids, and tannins as phytoconstituents. In the existing results, ethanolic extract inhibits clonidine induce catalepsy wherein the cataleptic impact of clonidine is mediated by histamine discharge from mast cells that onsets the inflammatory condition. Besides this inflammatory mediators, the subsequently parenteral administration of milk leads to stressed condition mediated by increases leucocytes count which can be stabilized by administration of an anti-stress or adaptogenic drug. In addition to a reduction in eosinophil count the ethanolic extract protected mast cells from degranulation it also significantly relaxed the ileum muscle strips that was precontracted by histamine which proposes the engrossment of β2 agonism in the relaxation of the tissue. This envisages a promising role in the asthmatic condition or allergic disorders. Thus it can be concluded, 70% ethanolic extract of seeds of the plant *Caesalpinia bonducella* (*Caesalpinia*ceae) is effective in the prophylaxis management of asthma. These activities justify the traditional use of this plant in the treatment of bronchoconstrictive diseases. An additional detailed study needs to be progressed to evaluate the clinical efficacy, isolation of the active ingredient and further toxicity study may warrant the development of the plant extract into proper drug or a dosage form. In future isolation and identification of phytochemicals, *in-vivo* studies are essential for a better understanding of their mechanism of action.

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Conflicts of interest:

The authors declare that they have no conflict of interest.

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